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INFLUENCE OF ROUTE AND PATTERN OF EXPOSURE ON THE PHARMACOKINETICS AND HEPATOTOXICITY OF CARBON TETRACHLORIDE

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ABSTRACT

Presently, there are many uncertainties in risk assessment of volatile organic compounds (VOCs), due to a paucity of data relevant to human exposure situations. It is unclear whether the relatively large VOC inhalation toxicity data base can be used qualitatively or quantitatively to forecast the consequences of ingestion of the chemicals in food or water. It is also unclear whether the existing VOC oral toxicity data base, which largely consists of bolus gavage studies, is applicable to drinking water hazard evaluation. The objective of our study was to evaluate the influence of route and pattern of exposure on the pharmacokinetics and target organ toxicity of carbon tetrachloride (CCl_4). Male Sprague-Dawley rats of 350-400 g inhaled 100 or 1000 ppm CCl_4 for 2 hr through a one-way breathing valve. Equivalent oral CCl_4 doses of 18.9 and 186 mg/kg were given to other groups of rats in an aqueous emulsion by oral bolus gavage or by constant gastric infusion over 2 hr. Serial blood samples were taken from the unanesthetized animals by means of an indwelling arterial cannula and analyzed for CCl_4 by headspace gas chromatography. Blood and liver samples were taken 24 hr post dosing for measurement of serum and hepatic microsomal enzymes. Gastric infusion resulted in a lower peak blood concentration (C-MAX) and area under the blood concentration versus time curve (AUC), but a greater hepatic cytochrome P-450 loss at the high dose than did inhalation. C-MAX, AUC, and hepatotoxicity indices were substantially higher for the oral bolus than the corresponding gastric infusion and inhalation groups. These findings demonstrate that the pattern and route of exposure can significantly influence the pharmacokinetics and acute hepatotoxicity of CCl_4. (Supported by U.S. Air Force Grant AFOSR 88-0277 and U.S. EPA Cooperative Agreement CR-812267.)

INTRODUCTION

A variety of volatile organic compounds (VOCs) have been identified as contaminants of drinking water supplies in the United States [1,2]. One class of contaminants of particular concern at present is the short-chain aliphatic halocarbon. In order to set standards which will protect against adverse health effects from drinking such water, reliable data on the toxic potential of each chemical are necessary. As the majority of prior interest in health hazards of halocarbons has centered around exposures in occupational settings, toxicological knowledge is largely based upon situations and experiments involving inhalation exposure. It is unclear, however, whether the results of inhalation studies can be used to accurately predict the consequences of ingestion of the chemicals.

There are very few applicable pharmacokinetic or toxicologic data sets from which to judge the validity of route to route extrapolations. The National Academy of Sciences [3] avoided the use of inhalation data in risk assessments of drinking water contaminants. They reasoned that the disposition and ensuing bioeffects of inhaled chemicals may differ markedly from that which occurs when the compounds are ingested. It was concluded that while inhalation studies may be of value from a qualitative standpoint, such studies may be of limited utility quantitatively in predicting consequences

of ingestion of many chemicals. In contrast, the U.S. Environmental Protection Agency (EPA) has applied the Stokinger-Woodward model [4] to inhalation data to derive guidelines for oral exposure to halocarbons. This means of direct route to route extrapolation was used to derive acceptable daily intake (ADI) values for a series of halocarbons, inducing trichloroethylene, 1,1,1-trichloroethane 1,2-dichloroethane, 1,1-dichloroethylene, and tetrachloroethylene, that were published in the Federal Register [5]. Such route to route extrapolations are based on two basic assumptions: (a) pharmacokinetics of toxicants are not influenced significantly by route of exposure; (b) target organ toxicity is independent of route of exposure.

Another important question is the applicability of results of oral bolus toxicity studies to health effects assessments of food and drinking water contaminants. Chemicals are usually administered as a single bolus in toxicity studies. Actual human exposures are quite different, since people normally consume relatively low levels of chemicals in food and water in divided doses over the course of a day. Therefore, another major objective of the current investigation was to determine the influence of dosage regimen on the pharmacokinetics and toxicity of an ingested halocarbon.

Carbon tetrachloride (CCl_4) was chosen for this investigation, since CCl_4 is a common water pollutant, as well as a potent hepatotoxin and hepatocarcinogen [6,7]. CCl_4 has been widely used in the manufacture of chlorofluorocarbons, in the fumigation of grain, in various industrial solvents, and in cleaning agents. CCl_4 also is produced as a by-product of the manufacture of a number of other chlorinated materials. Production of CCl_4 was approximately 600 million lbs. in 1983, although its use has steadily declined since 1974. Nevertheless, humans may still be exposed to CCl_4 in some occupational settings and in the environment. CCl_4 is often found as a contaminant of ground water and surface waters around hazardous waste disposal sites [7].

Specific aims of the current investigation were: (a) to characterize the pharmacokinetics of equivalent inhaled and ingested doses of CCl_4 over the same time-frame; (b) to contrast the hepatotoxicity of equivalent oral and respiratory doses of CCl_4; and (c) to assess the influence of different patterns of ingestion of CCl_4 on the relative bioavailability and hepatotoxicity of the chemical.

METHODS

Chemicals. Analytical-grade (99.9% pure) CCl_4 was obtained from J.T. Baker Chemical Company (Phillipsburg, NJ). All other chemicals and biologicals were purchased from Sigma Chemical Company (St. Louis, MO) and Aldrich Chemical Company, Inc. (Milwaukee, WI).

Animals and treatment. Male Sprague-Dawley rats were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). The animals were maintained on a 12-hr light/dark cycle, with light from 0600 to 1800 h and darkness from 1800 to 0600 h. The animals were randomly assigned to groups

and housed in stainless-steel cages in a ventilated animal rack. Purina Rodent Chow 5001® (Ralston Purina Co., St. Louis, MO) and tap water were provided ad libitum. The rats were used after a 2-week acclimation period, at which time their body weight ranged from 350-400 g. Chemical exposures were performed at approximately the same time each day (1000 to 1200 h). At least 24 hr before dosing, each rat was surgically prepared with an indwelling carotid artery cannula and gastric cannula. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of Ketamine HCl (100 mg/ml): Acepromazine maleate (10 mg/ml): Xylazine HCl (20 mg/ml) in a proportion of 3:2:1(V:V:V). The cannulas were exteriorized at the back of the neck through a harness, which allowed the animals relative freedom of movement, but prevented them from disturbing their cannulas.

CCl_4 inhalation and ingestion exposures. The inhalation exposure system was prepared as described by Dallas et al. [8,9]. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneva, Switzerland). A miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO) was attached to the face mask so that the valve entry port was directly adjacent to the nares of the test animal. CCl_4 inhalation exposures were initiated only after stable breathing patterns were established. One hundred ppm or 1000 ppm of CCl_4 was inhaled for 2 hr, and the respiration of each animal was continuously monitored. The minute volumes for the 100 and 1000 ppm groups, calculated by averaging the measurements taken at 5- to 15-min intervals during the 2-hr exposure, were 192.2 ± 17.8 and 209.8 ± 19.1 ml/min, (mean ± SE), respectively.

The administered dose for the inhalation exposures was estimated using the following equation: 0.5 x minute volume x time x inhaled concentration. The anatomic, valvular and mask deadspace totaled approximately 50% of the rat's tidal volume. It was assumed that CCl_4 residing in this deadspace did not participate in gas exchange. Thus, the factor of 0.5 represented the percentage of inhaled CCl_4 thought to reach the alveoli and be available for systemic absorption. The administered doses for the 100 and 1000 ppm exposures were calculated to be 13.9 and 186 mg/kg bw, respectively. These two doses were given orally as an aqueous Emulphor® emulsion, either by bolus gavage or by constant intragastric infusion, in a total volume of 5 ml/kg bw. All solutions were kept chilled, and the actual concentration of CCl_4 determined prior to dosing by gas chromatograph headspace analysis. Gastric infusions were carried out using gas-tight Hamilton® glass syringes mounted on a Harvard Microinfusion® pump calibrated to deliver accurate volumes at a consistent, predetermined rate. The infusions were carried out for 2 hr through the gastric cannula. Each orally-dosed animal was maintained in a restraining tube for the 2-hr period.

Blood sampling and CCl_4 analysis. Blood samples were withdrawn from the arterial cannula via a 3-way stopcock into a 1-ml syringe. Serial 25-µl blood samples were taken at time intervals of 2 to 60 min for up to 12 hr during and following dosing. Each blood withdrawal was followed by a heparinized saline flush (10 U heparin/ml) of the same volume, in order to replace lost fluid volume. Blood samples from high-dose exposure groups were diluted in ice-cold saline solution, in order that they could be analyzed within the linear

range of the electron capture detector of the gas chromatograph (GC). Blood samples were quickly transferred to dry, ice-chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 auto-sampler unit of a SIGMA 300 GC (Perkin-Elmer, Norwalk, CT), where it was heated to 80°C and then injected automatically into the GC column for analysis.

Calculation of pharmacokinetic parameters. Blood concentration versus time profiles were evaluated by the LAGRAN [10] computer program for determination of relevent pharmacokinetic parameters.

The following pharmacokinetic parameters were calculated from the blood concentration versus time data:

$$t_{1/2} = \frac{0.693}{K}$$

where t½ is the elimination half life of CCl_4 and K is the terminal elimination rate constant.

$$CL_{oral} = \frac{Dose}{AUC}$$

where CL_{oral} is the apparent oral clearance and AUC is the area under the blood CCl_4 concentration versus time curve from time zero to time infinity, as calculated by the Lagrange method.

Clinical chemistry and hepatic microsomal analyses. Twenty-four hr after dosing, each animal was anesthetized with ether and blood collected by cardiac puncture into evacuated serum separation tubes (SST®, Becton Dickinson, Rutherford, NJ). The blood was kept on ice to allow it to coagulate and then centrifuged. The serum samples were transferred to polypropylene microcentrifuge tubes and stored in a freezer at -80°C prior to analysis. The conversion of NADH to NAD over time was used to measure sorbitol dehydrogenase (SDH) activity [11] and glutamic-pyruvic transaminase (GPT) [12] activity in serum. The liver was perfused in situ with chilled normal saline. Liver samples were taken, and the hepatic microsomal fraction was prepared from the 9000 g supernatant, suspended in TRIS-KCl buffer containing 20% glycerol, 1 mM DTT and 1 mM EDTA, and stored at -80°C. The protein concentration in the hepatic microsomes was determined by the method of Lowry et al. [13]. Hepatic microsomal cytochrome P-450 levels were measured by the method of Omura and Sato [14].

Statistical analysis. The statistical significance of differences between inhalation and corresponding ingestion groups, and oral bolus and corresponding gastric infusion groups was evaluated by Student's t test, with $p < 0.05$ set as the minimum level of significance.

RESULTS

The arterial blood concentration versus time profiles in rats during and following inhalation of 100 or 1000 ppm CCl_4 for 2 hr are presented in Figure 1. CCl_4 was rapidly absorbed from the lung, in that substantial levels of CCl_4 were detected in the arterial blood at the initial sampling time (5 min), and near steady-state was soon achieved. The curves were asymptotic, in that they continued to gradually rise throughout the 2-hr exposure period. The increase in the inhaled concentration from 100 to 1000 ppm produced a proportional increase (i.e., 10-fold) in the near steady-state blood concentrations. Upon cessation of CCl_4 inhalation, the blood levels initially fell very rapidly, then diminished at a slower rate during the 10-hr post-exposure monitoring period. The terminal elimination half-lives (t½) were comparable for the two exposure levels (Table 1). Clearance appeared to be somewhat lower at the higher exposure level, but values for the two groups were not significantly different. The total administered doses were determined to be 18.9 and 186 mg CCl_4/kg bw for the 100 and 1000 ppm groups, respectively.

Parallel blood concentration versus time profiles were manifest in animals following administration of 18.9 and 186 mg CCl_4/kg as an oral bolus (Figure 2). CCl_4 was readily absorbed from the gastrointestinal (GI) tract. Peak concentrations of CCl_4 in the blood (C-MAX) were reached within 10 min after gavage at both dosage-levels. The blood levels decreased more slowly than in the inhalation groups in the initial minutes post dosing, indicative of continuing absorption during this period in the orally-dosed animals. The t½ values of 239 and 233 min for the 18.9 and 186 mg/kg groups, respectively, were quite similar to the t½ values for inhalation exposure (Table 1).

The pattern of systemic uptake and elimination of CCl_4 is quite different from inhalation when the 18.9 and 186 mg/kg doses are given by constant gastric infusion. Blood levels increase steadily over the entire 2-hr exposure period, and then decline at a relatively slow, constant rate post-exposure (Figure 3). The curves for the 18.9 and 186 mg/kg groups appear to be parallel, but close inspection of the pattern of uptake reveals some disparity during the 2-hr administration period. Although blood concentrations generally are directly proportional to dose in the inhalation and oral bolus groups, there is a 35-fold increase in blood concentration with the 10-fold increase in dose during the initial minutes of gastric infusion. The blood levels remain relatively low for the first 20-30 min of the infusion in the low-dose animals, then increase steadily. In contrast, the uptake curve in the high-dose infusion animals more nearly resembles that for inhalation exposure (i.e., relatively rapid attainment of near steady-state).

The effect of pattern of ingestion of CCl_4 on selected pharmacokinetic parameters can be seen in Table 1. Maximum blood levels were about 5 to 6 times higher following oral bolus dosing than during gastric infusion. These differences are readily apparent when the blood levels are plotted on a linear scale (Figure 4). The oral bolus groups had significantly greater AUC values than corresponding gastric infusion groups. Clearance, therefore, was significantly increased in the rats receiving CCl_4 by constant infusion. The rate of clearance was particularly high in the low-dose infusion group.

The influence of route of exposure on CCl_4 pharmacokinetics can also be seen in Table 1 and Figure 4. Throughout the 2-hr exposure period concentrations of CCl_4 in arterial blood were significantly higher in animals inhaling CCl_4 than in animals receiving it by gastric infusion. As a result, AUC values were higher for the inhalation exposures, though intersubject variability precluded a statistically significant difference at the high dosage-level. Clearance was significantly greater and t½ shorter in rats receiving the low dose by gastric infusion than in the corresponding inhalation group.

The magnitude of CCl_4 hepatotoxicity was more affected by pattern than by route of CCl_4 exposure. Inhalation of 100 ppm CCl_4 or gastric infusion of the equivalent administered dose (i.e., 18.9 mg/kg) had relatively little effect on SDH and GPT activities. Rats receiving 18.9 mg/kg as a single oral bolus, however, exhibited a significant elevation of each serum enzyme. The high dosage-level (i.e. 1000 ppm/186 mg/kg) produced comparable increases in enzyme levels in the inhalation and gastric infusion groups. Three- to four-fold greater increases in SDH and GPT occurred in response to administration of 186 mg/kg as a single oral bolus. The low dosage-level appeared to cause modest reductions of comparable magnitude in all 3 groups in hepatic microsomal cytochrome P-450, though the decreases were not sufficient to be statistically significant. There were significant reductions in P-450 in animals receiving the high dose of CCl_4 by gastric infusion or as an oral bolus.

DISCUSSION

Delineation of the relative pharmacokinetics and toxicity of inhaled and ingested VOCs requires innovative approaches. Physiologically-based pharmacokinetic (PBPK) models are finding increasing use in predicting the dynamics of VOCs in blood and selected tissues under different exposure conditions. Paustenbach et al. [16], for example, describe a PBPK model which adequately forecasts the kinetics of CCl_4 and its metabolites for varying inhalation scenarios. Data from direct measurements of VOC levels and toxic effects in animals are required, nevertheless, to validate and adjust PBPK models to improve their accuracy. Results of direct measurement studies reported to date, in which comparable doses of VOCs have been given orally and by inhalation [17-21], are of limited utility in route to route extrapolation. Most such studies have involved administration of the oral dose as a single bolus by gavage and inhalation exposure over a period of hours. VOCs, as a chemical class, are quite rapidly absorbed following oral dosing, and then quickly eliminated from the bloodstream. Prolonged inhalation of VOCs, in contrast, serves as a constant infusion into the systemic circulation. A more logical way to directly determine the influence of exposure route on VOC disposition would be to give equivalent doses over the same time-frame intragastrically and by inhalation. This is the experimental approach employed in the current investigation.

On the basis of physiological and anatomical considerations, it would appear that the route of exposure should significantly influence the quantity of chemical reaching a particular target tissue and the resulting degree of toxic effect on the tissue. The lung is an optimal site for systemic absorption

because of its large surface area, intimate alveolar-capillary interfaces and high rate of blood perfusion. As VOCs are small, uncharged, lipophilic molecules, they are rapidly absorbed from the lung into the systemic circulation [22]. Compounds absorbed into the pulmonary circulation are transported via the arterial blood directly to tissues of the body, without first having to pass through an eliminating organ. The GI tract is also well suited for absorption of VOCs, though its total surface area is less than that of the lung, and it receives only a fraction of the total cardiac output. Also, VOCs absorbed from the GI tract into the portal blood are subject to "first pass" elimination by the liver and lungs. In the current study, CCl_4 was rapidly absorbed from both the lung and GI tract, in that substantial levels of CCl_4 were detected in the arterial blood of the inhalation and oral bolus groups at the earliest sampling times. A substantial proportion of the low dose (18.9 mg/kg) did not reach the systemic circulation in the gastric infusion group, as reflected by significantly lower AUC and C-MAX values in this than in the corresponding inhalation group. A similar pattern was seen at the high dose (186 mg/kg), though the difference between the gastric infusion and inhalation AUCs was not statistically significant. Thus, it appears that a significant amount of CCl_4 absorbed from the GI tract will be metabolized by the liver and/or exhaled before reaching the arterial circulation and extrahepatic organs. First-pass elimination will be most efficient at low doses of CCl_4, since the liver's capacity to metabolize the chemical is limited [16]. Also, in high doses, CCl_4 can cause liver injury and thereby inhibit its own metabolism [23].

If ingested CCl_4 undergoes extensive first-pass extraction by the liver, it is logical to assume that the liver will accumulate a higher dose and experience more pronounced injury than following an equivalent inhalation exposure. The aforementioned arterial CCl_4 concentration-time data support this assumption, though it will be necessary to measure the concentration of CCl_4 in the liver over time (i.e., the tissue dose), in order to ascertain the relative contribution of the liver and lungs to presystemic elimination. The cytochrome P-450 data in Table 2 suggest that ingested CCl_4 is more hepatotoxic than inhaled CCl_4. These and the serum enzyme data are not conclusive, however, due to pronounced intersubject variability. Thus, the study is being repeated to clarify whether the liver is actually at greater risk of injury upon oral exposure to CCl_4. As cumulative uptake (i.e., the systemically absorbed dose) can be measured during inhalation exposures, both the "absorbed" dose and the "administered" dose for inhalation will be given to rats within the same time-frame by gastric infusion. Blood and liver CCl_4 levels, as well as a variety of indices of hepatotoxicity, will be monitored over time, in order to more adequately assess the influence of route of exposure on target organ toxicity.

Although VOCs such as CCl_4 are commonly given by gavage as a single bolus in oral toxicity studies, human exposures to VOCs in the atmosphere and in drinking water typically occur on a repetitive, or continuing basis as a person inhales air or consumes water over the course of a day. The relatively small quantities of chemicals absorbed upon repetitive ingestion may be readily metabolized and eliminated, so that toxic levels are not reached in the blood and target organs. Single high doses are more likely to exceed a toxic threshold and produce injury. Our study demonstrates that administration of CCl_4 as an oral bolus results in C-MAX and AUC values which are

substantially greater than when the same doses are given over 2 hr by gastric infusion or inhalation. As expected, CCl_4 is significantly more hepatotoxic when given as a single oral bolus. Chloroform ($CHCl_3$) and a number of other halocarbons have been found to produce a high incidence of hepatocellular carcinoma, when given to B6C3F1 mice in corn oil by gavage. Jorgenson et al. [24], however, saw no evidence of hepatic tumorigenesis when these mice were given the same doses of $CHCl_3$ in drinking water. Similarly, Klaunig et al. [25] found that $CHCl_3$, 1,1-dichloroethylene and 1,2-dichloroethane were not carcinogenic when given to mice in their drinking water, although each halocarbon has been reported to be a hepatocarcinogen when given daily as a single oral bolus. Thus, results of oral bolus studies may not accurately represent/predict risks of environmental exposure to halocarbons and other VOCs in drinking water and air.

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Table 1

Influence of Route and Pattern of Exposure on CCl_4 Pharmacokinetic Parameters[a]

Exposure Route/ Pattern	Dose	AUC ($\mu g \cdot min/ml$)	C-MAX ($\mu g/ml$)	$t_{1/2}$[b] (min)	CL_{oral}[c] (ml/min/kg)
Inhalation	100 ppm	215 ± 17[e]	1.14 ± 0.09[e]	262 ± 19	92.0 ± 7.7[e]
	1000 ppm	2732 ± 300[e]	16.80 ± 1.93[e]	233 ± 27	73.0 ± 9.1[e]
Oral Bolus	18.9 mg/kg	348 ± 50[d]	3.29 ± 0.28[d]	239 ± 18	60.6 ± 7.6[d]
	186 mg/kg	4995 ± 699[d]	38.96 ± 2.55[d]	233 ± 31	42.6 ± 6.7[d]
Gastric Infusion	18.9 mg/kg	145 ± 10[d,e]	0.61 ± 0.07[d,e]	166 ± 18[d,e]	132.5 ± 9.2[d,e]
	186 mg/kg	2161 ± 166[e]	6.15 ± 0.92[d,e]	185 ± 32	89.0 ± 6.5[e]

[a] Values are mean ± SE for 5-8 rats.
[b] Harmonic mean half-lives, SE estimated by the jackknife technique of Lam et al. [15].
[c] Apparent oral clearance (CL_T/F).
[d] Significantly different from corresponding inhalation group at $p < 0.05$.
[e] Significantly different from corresponding oral bolus group at $p < 0.05$.

Table 2

Influence of Route and Pattern of Exposure on Serum Enzyme Activities and Hepatic Microsomal Parameters[a,b]

Exposure Route/ Pattern	Dose	SDH (mU/ml)	GPT (mU/ml)	Hepatic Microsomal Cytochrome P-450 (nmol/mg protein)
Control	0	4.8 ± 0.6	23.0 ± 3.1	0.58 ± 0.10
Inhalation	100 ppm	8.6 ± 1.7	34.7 ± 1.9[c,e]	0.43 ± 0.05
	1000 ppm	92.2 ± 63.7[c]	130.6 ± 61.5[c,e]	0.31 ± 0.05[c]
Oral Bolus	18.9 mg/kg	34.7 ± 12.4[c]	57.3 ± 11.5[c,d]	0.40 ± 0.06
	186 mg/kg	342.7 ± 169.5[c]	516.5 ± 181.6[c,d]	0.14 ± 0.03[c,d]
Gastric Infusion	18.9 mg/kg	6.8 ± 1.1	18.0 ± 1.6[e]	0.40 ± 0.02
	186 mg/kg	98.3 ± 14.1[c]	162.9 ± 41.2[c,e]	0.19 ± 0.03[c]

a Rats of 350-400 g were inhaled 100 or 1000 ppm of CCl_4 for 2 hr. Other groups of rats were given a dose of 18.9 or 185 mg/kg in an aqueous emulsion by gavage as a bolus or by gastric infusion over a 2-hr period. All rats were sacrificed 24 hr post exposure.

b Values are expressed as mean ± SE for groups of 4-8 rats.

c Significantly different from control group at $p < 0.05$.

d Significantly different from corresponding inhalation group at $p < 0.05$.

e Significantly different from corresponding oral bolus group at $p < 0.05$.

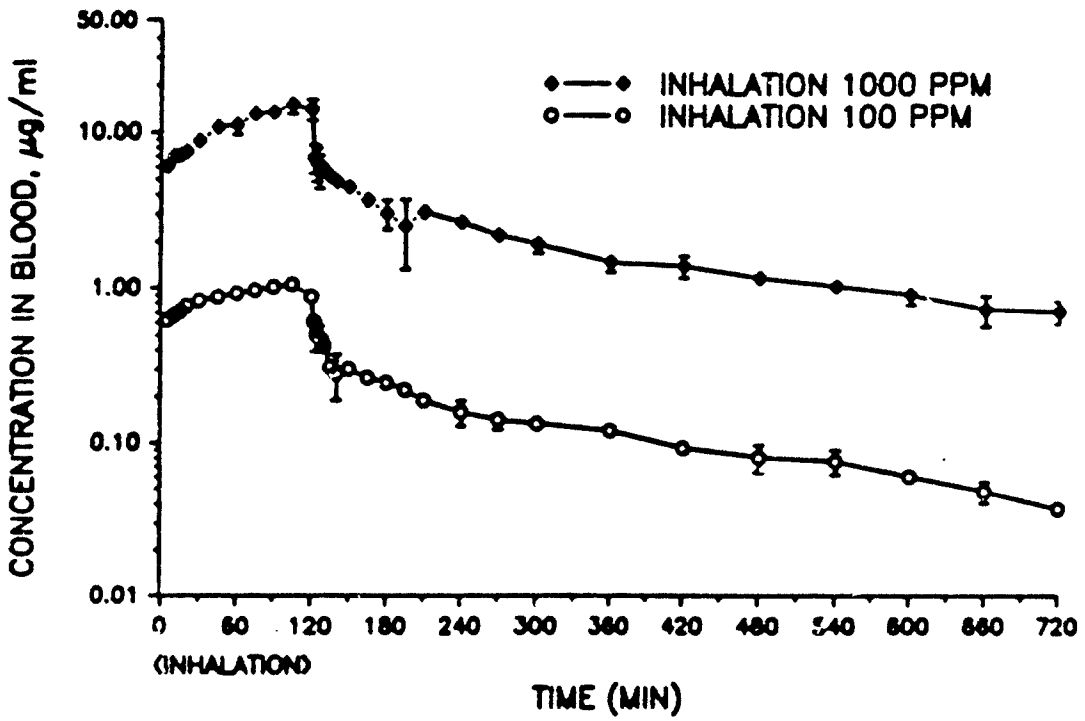

Figure 1. Blood CCl_4 concentration versus time profiles in rats during and following inhalation of 100 or 1000 ppm CCl_4 for 2 hr. Arterial samples were taken at 2- to 60-min intervals. Values are mean ± SE for 6-8 rats. SE bars are not included for some groups due to small SE.

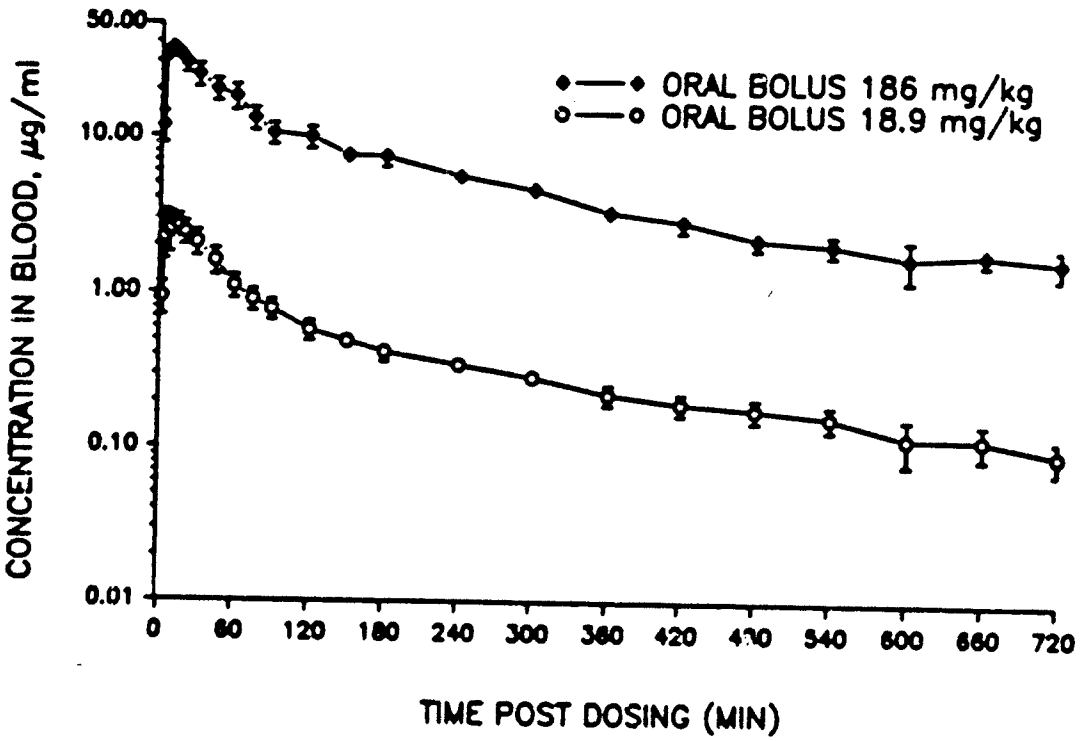

Figure 2. Blood CCl_4 concentration versus time profiles in rats following administration of an oral bolus of 18.9 or 186 mg CCl_4/kg by gavage. Arterial samples were taken at 2- to 60-min intervals. Values are mean ± SE for 7-8 rats.

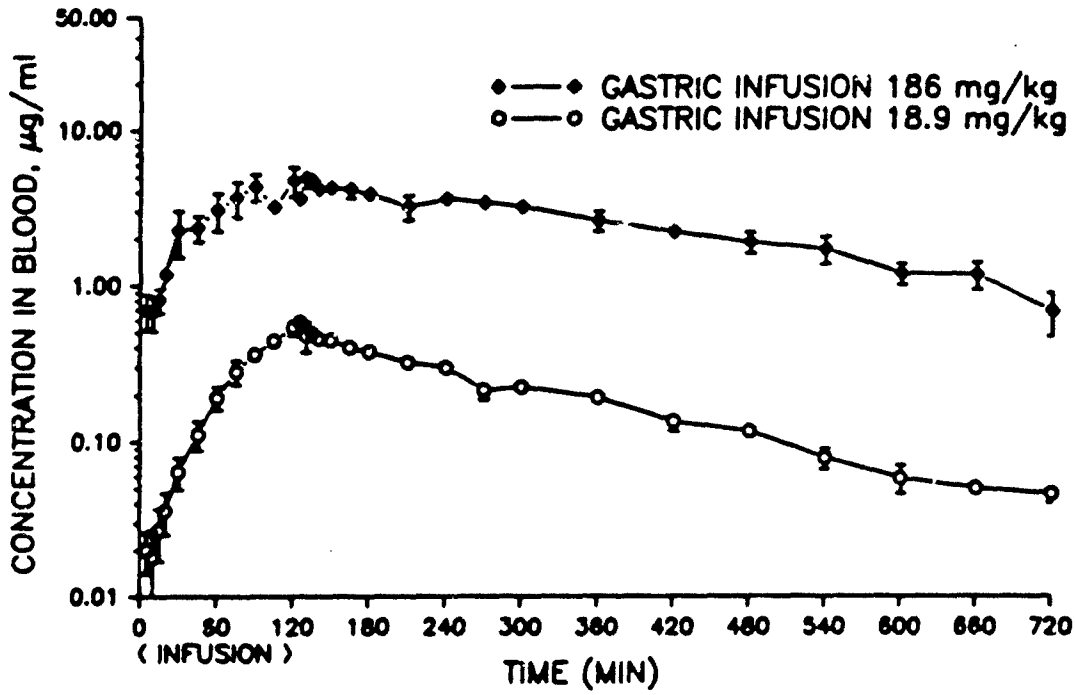

Figure 3. Blood CCl_4 concentration versus time profiles in rats during and following constant gastric infusion of 18.9 or 186 mg CCl_4/kg for 2 hr. Arterial samples were taken at 2- to 60-min intervals. Values are mean ± SE for 5-8 rats.

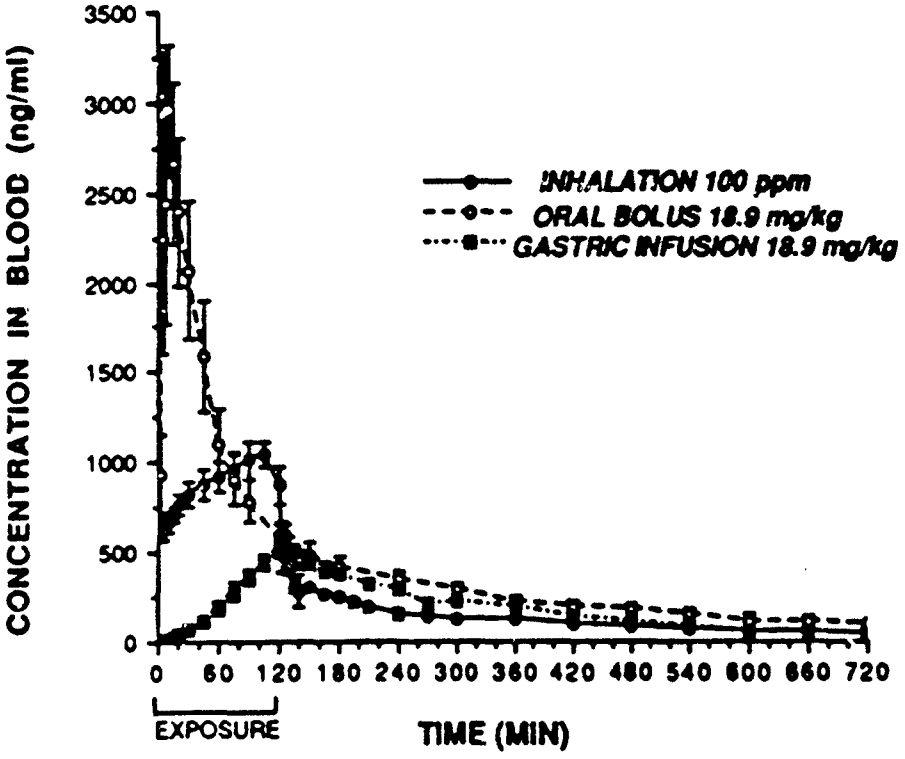

Figure 4. Effect of route and pattern of exposure on blood CCl_4 concentration-time profiles. Rats inhaled 100 ppm of CCl_4 for 2 hr. An equivalent oral dose of 18.9 mg/kg was given in an aqueous emulsion either by gavage as an oral bolus and by constant gastric infusion for 2 hr. Arterial CCl_4 concentrations were measured at 2- to 60-min intervals. Values are mean ± SE for 5-8 rats.